Studies on the *Mytilus edulis* Community in Alamitos Bay, California. VI. Regulation of Anaerobiosis by Dissolved Oxygen Concentration

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(2 Text figures)

IT HAS BEEN RECOGNIZED for some time now that many marine and brackish water bivalves are capable of withstanding oxygen poor environments for prolonged periods of time (HOCHACHKA *et al.*, 1973). In a previous paper, REISH & AYERS (1968) examined the effect of chlorinity and reduced dissolved oxygen (D. O.) concentrations upon byssal thread production by *Mytilus edulis* Linnaeus, 1758, from the Alamitos Bay community. It was noted that dissolved oxygen concentrations down to 0.9 mg/*l* had little effect on byssal thread output, a parameter indicative of overall organismal metabolism. These findings therefore gave some indication of the bay mussel's ability to cope with environments poor in oxygen.

Numerous biochemical and physiological studies have examined mechanisms by which *Mytilus edulis* and several other bivalves tolerate prolonged periods of anoxia (GILLES, 1970; HAMMEN, 1969; MALANGA & AIELLO, 1972; MUSTAPHA & HOCHACHKA, 1973b; DE ZWAAN *et al.*, 1973). Previous work indicates that the bay mussel and its metabolic allies (*Crassostrea, Rangia, Modiolus*) are capable of sustaining energy (in the form of ATP) production during anoxia by diverting carbohydrate energy resources away from oxygen dependent energy-yielding pathways (such as the Krebs cycle). One of the pivotal reactions implicated in this strategy is catalyzed by a cytoplasmic enzyme, phosphoenolpyruvate carboxykinase (PEPCK). Regulation of this enzyme's catalytic properties by endogenous factors has been well documented (MUSTAFA & HOCHACHKA, 1973a; DE ZWAAN & DE BONT, 1975). Very little is known concerning the effect of exogenous (environmental) factors, such as dissolved oxygen concentration upon the PEPCK reaction. We have, therefore, examined the role played by D. O. concentration in modifying the activity of this enzyme, to better understand the adaptive significance of the PEPCK reaction in the bay mussel.

MATERIALS AND METHODS

Mytilus edulis was collected from boat dock pontoons in Alamitos Bay, Long Beach, California. Immature specimens, 15 - 20 mm in width and weighing 3 - 5 g, were used to minimize the metabolic effects of sexual maturity. Mussels were held for one week at 13±1° C in natural sea water with a salinity of 34‰ prior to experimentation. Mussels were then placed in individual stoppered 500 m*l* Erlenmeyer flasks containing 150 m*l* sea water and subjected to reduced dissolved oxygen concentrations. Oxygen reduction within the flask was produced by the nitrogen injection technique of REISH & RICHARDS (1966). A Beckman Fieldlab analyzer was used to determine the dissolved oxygen concentration of experimental sea water.

Mussels were sacrificed for enzyme activity on days 1 through 8 following nitrogen injection. Since the nitrogen injection technique produces a slow decline in sea water D. O. levels for 48 hrs, followed by a leveling off of D. O.

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concentration at a given constant value thereafter, these procedures permitted the monitoring of enzyme activity as D. O. concentration gradually declined. Enzyme activities in mussels sacrificed on subsequent days therefore reflect the combined effects of exposure to declining D.O. concentrations, followed by varying lengths (in days) of exposure to a constant level of reduced D. O. concentration. The final reduced D. O. concentrations studied were 5.6, 3.2, 2.3 and 1.0 mg/*l*.

PEPCK activity was assayed by a modification of the spectrophotometric technique described by Utter & Kurahashi (1954). Posterior adductor muscle was used exclusively since this tissue exhibits maximal PEPCK activity in *Mytilus edulis* (de Zwaan & van Marrewijk, 1973). All assays were performed at 25° C. Enzyme activity is expressed as micromoles of NADH oxidized/min/mg protein = one unit (U) of enzyme activity. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951).

RESULTS

The effect of reduced D. O. concentrations on PEPCK activity in adductor muscle tissue of the bay mussel is graphically presented in Figure 1. PEPCK activity varied inversely with D. O. concentration down to, and including, 2.3 mg/*l*. Below this level, PEPCK activity was suppressed. Figure 2 graphically depicts the increase in adductor muscle PEPCK activity over time, during exposure to reduced D. O. concentrations which declined to a constant level of 2.3 mg/*l*. *Mytilus edulis* rapidly responded to declining D. O. concentrations in that maximal PEPCK activity was produced shortly after (within 24 hours) D. O. concentrations reached minimum levels. This maximal level of enzyme activity was exhibited throughout the remaining 5 days of exposure of 2.3mg/*l* D. O. concentration.

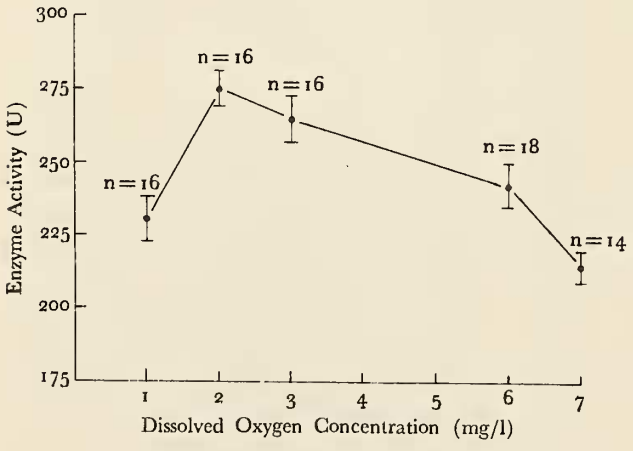

Figure 1

Effect of 6 Days of Exposure to Reduced Dissolved Oxygen Concentration on PEPCK Activity in Posterior Adductor Muscle Tissue of *Mytilus edulis*. Values are given as the mean of n samples with corresponding standard deviations represented as vertical bars

DISCUSSION

Several studies have shown enzymes implicated in anaerobic energy production by invertebrates to be regulated by environmental oxygen concentration (Beattie, 1971; Cripps & Reish, 1973; Hammen & Lum, 1966). These data represent the first report of such findings in *Mytilus edulis*. Significant changes in enzyme activity were produced by prolonged (8 days) exposure to a series of experimentally reduced D. O. concentrations, all of which are known to occur in Alamitos Bay (Reish, 1964; Reish & Moore, 1969).

Previous studies dealing with the regulation of anaerobiosis in *Mytilus edulis* have correlated such adaptations to anaerobic conditions produced by valve closure in response to aerial exposure (Mustapha & Hochachka, 1973a; de Zwaan & Zandee, 1972). Consequently, they have failed to examine the functional role of anaerobic adaptations during submergence. Many populations of *M. edulis*, including the one used in this study, never experience aerial exposure conditions employed by many workers to induce anaerobiosis in this species. Yet *M. edulis* is capable of surviving many days in the presence of substantially reduced D. O. concentrations (14 days at D. O. levels below 0.9 mg/*l*) (Reish & Ayers, 1968). If one assumes that increased levels of PEPCK activity are indicative of energetically efficient anaerobic glucose degradation, the observed changes in PEPCK activity, in response to reduced D. O. concentrations, should help to explain how the bay mussel tolerates anoxic or hypoxic conditions in nature. It should be emphasized that the correlation between PEPCK activity and anaerobic energy production in bivalves is merely hypothetical, although this relationship is supported by abundant indirect evidence (Gilles, 1970; Saz, 1971; Zs-Nagy & Ermini, 1972).

Maximal PEPCK activity was produced by exposure to a D. O. concentration of 2.3 mg/*l*. Mussels held at D. O. concentrations above this level exhibited decreased enzyme activities, In D. O. concentrations above 2.5mg/*l* *Mytilus edulis* is an oxyregulator, that is, at a given temperature

M. edulis maintains its oxygen consumption at a constant rate over the range of environmental oxygen levels from 2.5mg/*l* to saturation (Bayne, 1971). From Figure 1 it is apparent that declining D. O. concentrations, down to a critical level, stimulate PEPCK activity. These results suggest that the PEPCK reaction plays an important role in carbohydrate metabolism, even under conditions suitable to maximal functioning of aerobic metabolism (oxygen consumption). These results are in agreement with recent kinetic studies which have shown that PEPCK can operate during aerobic conditions (de Zwaan & de Bont, 1975), and support the postulation of Livingstone & Bayne (1974) that both major pathways of intermediary carbohydrate metabolism (one mediated by pyruvate kinase and the other by PEPCK) can proceed together. A study of the effect of D. O. levels on the pyruvate kinase reaction would help shed some light on the predominance of one pathway over the other during anaerobic conditions.

The observed decrease in PEPCK activity associated with maintenance at a D. O. concentration of 1.0 mg/*l* was not expected. This oxygen level is within the range in which *Mytilus edulis* oxyconforms, or fails to regulate its oxygen consumption at a constant rate (Bayne, 1971). Thus, aerobic respiration decreases in direct relation to D. O. concentrations below 2.5 mg/*l*. As compensation for the decrease in energy production by oxygen dependent pathways, one would expect to see an increase in oxygen independent energy yielding metabolism (that mediated by the PEPCK reaction) at this oxygen level. Since *M. edulis* can survive for more than 14 days under these conditions, energy production is apparently coming from pathways not involving the PEPCK reaction, or *M. edulis* begins to decrease its overall metabolism (energy demands) at this D. O. concentration. The latter view is supported by work of Reish & Ayers (1968) in which an overall metabolism indicator, byssal thread output, decreased at D. O. concentrations below 1 mg/*l*. It is interesting to note that an environmental stress factor which significantly affects a subcellular metabolic process, PEP CK activity, did not yet influence an organismal level function such as byssal thread production.

Previous studies with *Mytilus edulis* have not dealt with the time course of enzymatic response to anaerobic conditions, even though a standard time period of valve closure (21 hr) has been developed to induce workable PEPCK activity levels (de Zwaan & Zandee, 1972). Data presented in Figure 2 clearly indicate the rapid manner in which *M. edulis* responds enzymatically to declining D. O. levels. As can be seen, maximal PEPCK activity was exhibited 24 hours after D. O. levels had

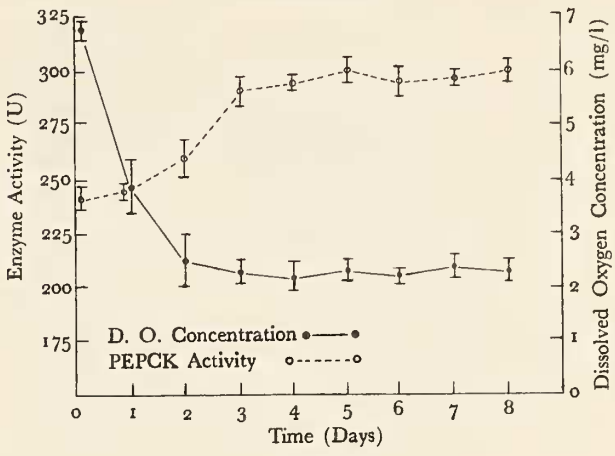

Figure 2

Increase in PEPCK Activity in Posterior Adductor Muscle of *Mytilus edulis* over Time in the Presence of Declining Dissolved Oxygen Concentrations. Each symbol represents the mean value for at least 5 samples with corresponding standard deviations represented as vertical bars

reached minimal values (2.3 mg/*l*) and had begun to respond to declining ambient oxygen levels within 48 hrs of exposure. Responses to declining oxygen tensions, as indicated by changes in the oxygen consumption of *M. edulis*, have been elicited within a one hour time period by Bayne (1971). It is apparent that the sub-cellular metabolic response to this environmental variable follows the tissue level response in the bay mussel. Of great interest is the dissimilarity, time-wise, between metabolic homeostasis achieved during thermal and during hypoxic stress. Results reported here indicate that homeostasis for anaerobiosis during hypoxia is reached within 48 hours of stress onset (declining oxygen levels), whereas Newell & Pye (1970) have shown that metabolic homeostasis during thermal acclimation required 7 - 9 days, presumably due to the time period required to develop isozymes appropriate to a particular temperature regime (Hochachka & Somero, 1968). The rapid onset of "steady state" anaerobiosis in *M. edulis*, coupled with its continuance over an 8 day period, suggests that isoenzymatic forms of certain enzymes involved in anaerobiosis may be absent in the bay mussel. More information is needed concerning the changes in pivotal enzyme kinetics during prolonged exposure to hypoxic conditions.

SUMMARY

1. The effect of dissolved oxygen (D. O.) concentration on the activity of an enzyme implicated in anaerobic energy production in *Mytilus edulis* was examined by a series of experiments in the laboratory.
2. Enzymatic response to declining D. O. concentrations occurred within 48 hrs.
3. Following 6 days of exposure to a constant D. O. concentration, enzyme activity varied inversely with D. O. concentrations down to and including 2.3 mg/*l*.
4. Enzyme activity was suppressed at a D. O. concentration of 1 mg/*l*.
5. The significance of these findings to the bay mussel's ability to withstand hypoxia is briefly discussed.

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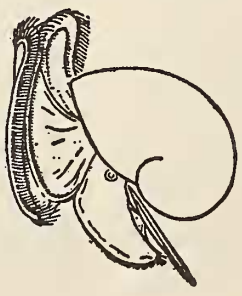

Marine Fouling Animals in the Bay of Hamana-ko, Japan

BY

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(4 Text figures)

INTRODUCTION

ALTHOUGH FOULING ANIMALS have harmful effects upon the submerged structure, they can be used as indicators of changing environmental characters in coastal and harbor waters. In farms which engage in oyster or pearl oyster culture by means of the raft system, the fouling community is generally a more important and abundant indicator than the benthic one. The large number of fixed rafts which are widely distributed in the Bay of Hamana-ko serve as suitable substrata for attachment of fouling organisms.

From November 1972 to November 1973 test pipes were exposed to obtain information on the abundance, horizontal or vertical distribution, and seasonality of attachment of fouling organisms in the waters of oyster farms in the Bay of Hamana-ko.

MATERIALS AND METHOD

The Hamana-ko is divided into 2 parts by a peninsula which extends from the northern coast southward into the central region of the bay; the western part is the main region and is called "Hon-ko," while the eastern part is known as the inlet of "Shonai-ko" (Figure 1). Test pipes were exposed at 5 stations; station A was located at the mouth of Hon-ko and the remaining 4 stations were staggered away from the mouth towards the innermost extension of Shonai-ko. Water depth of all stations was less than 3 m. Grey polyvinylchloride piping with an outer diameter of 5 cm was used in this study. Pipes were driven vertically into the sea bottom in order to collect a series of fouling organisms between the surface and the bottom layers. Three months after embedding, test pipes were removed from each station and replaced with new ones. In this manner, 5 test pipes which had been exposed for a 3-month period were collected 4 times at each station during the 1-year experimental study. Collected pipes were cut into sections at intervals of 30 cm between the mean tidal level and sea bottom and subsequently fouling organisms were stored in approximately

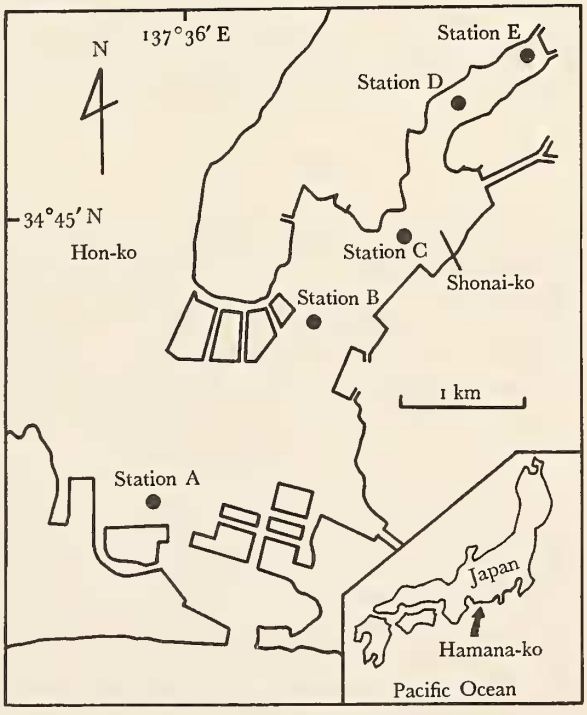

Figure 1

Map of the Bay of Hamano-ko and Location of Test Stations in Shonai-ko

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10% formalin in order to preserve them, pending observation and measurement.

Analyses included identification of sessile organisms, observation of attaching and covering order among dominant species. Subsequently, those fouling organisms attached on the upper 10 cm length of each 30 cm section were scraped from all test pipes. The fouling organisms from each station were classified into 12 groups, *i. e.*, Algae, Porifera, Hydrozoa, Actiniaria, Turbellaria, Polychaeta, Bryozoa, Bivalvia, Cirripedia, Amphipoda, Ascidiacea, and Miscellaneous.

The miscellaneous group was composed primarily of mud and amphipod tubes on November-February pipes, and fragments of seaweeds or animals on February-May pipes at all stations. Tentative identification of principal fouling animals was made by the authors, and specific identifications of tubeworms and barnacles were conducted by two specialists. The wet weight of each species or group was determined separately after removal of excess liquid with a paper blotter.

Observations of sea conditions, water temperature, chlorinity and dissolved oxygen at surface and at bottom layers, and transparency were carried out monthly from November 1972 to November 1974 at each station. In this paper results of observations from February 1973 to January 1974 were used for analyses of sea conditions.

RESULTS

Sea Conditions at Exposure Stations: The amplitudes of seasonal variations in temperature, chlorinity and dissolved oxygen at the surface and bottom layers increased gradually with distance away from the mouth of Hon-ko to the inner part of Shonai-ko. The surface temperature fluctuated seasonally from 7°C to 27°C at station A, and from 2° C to 33° C at station E; and the bottom temperature from 7°C to 27°C at station A, and 6°C to 31°C at station E. At the mouth of Hon-ko, the monthly temperature change at the surface almost coincided with that at the bottom throughout the year, due to good mixing caused by a strong tidal current in this area. However, in Shonai-ko it is suggested that thermoclines were formed during summer and winter based upon marked differences in temperature between surface and bottom water. The chlorinity of both layers changed seasonally from 16.0‰ to 18.8‰ at station A, and from 8.6‰ to 16.0‰ at station E. In Shonai-ko, monthly changes in chlorinity of both layers appeared to fluctuate in parallel with each other, and the surface chlorinity was consistently lower than that of the bottom. The above mentioned observations suggest that the changing rate of sea water in this area was small and monthly fluctuations of chlorinity were mainly controlled by fresh water flowing from rivers.

Monthly changes in dissolved oxygen of both layers at all stations displayed comparatively smooth concave curves exhibiting a depression in summer. Irregular increase or decrease of dissolved oxygen which occurred from May to October seems to be related to the appearance or disappearance of red tides which frequently occurred from early summer through fall in Shonai-ko. Unfortunately, however, knowledge regarding the influence of low dissolved oxygen on fouling animals is nonexistent.

Water conditions at station A probably varied according to those of open coastal water running into the bay. In view of the differences in the CL-T diagrams shown for each station in Figure 2, it is suggested that open

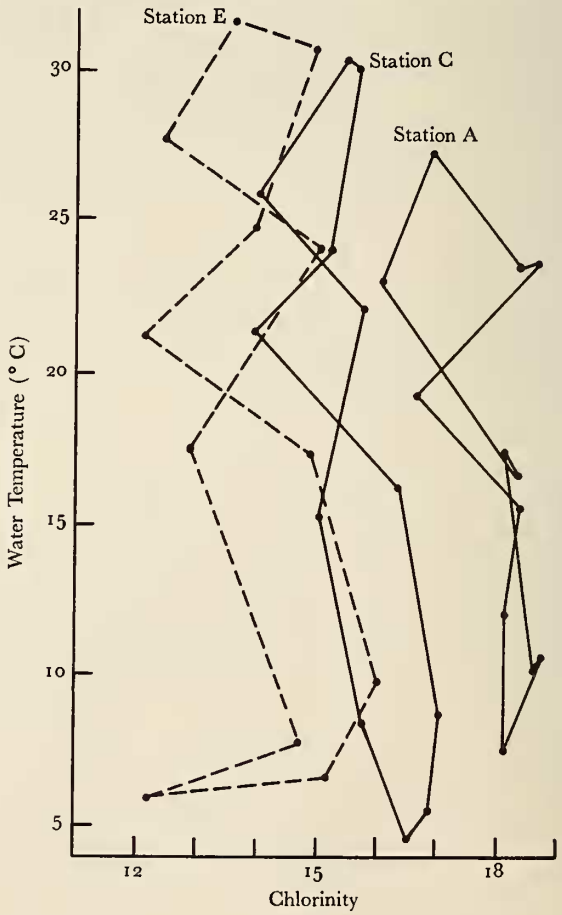

Figure 2

Chlorinity - Temperature Diagrams of Bottom Water Layers at Stations A, C, and E